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Effect of collection areas, growth stage and fruit body parts on the nutrient and antioxidant contents of *Termitomyces letestui* (Lyophyllaceae) from Cameroon

Choumessi T. Aphrodite[1], Njouonkou André-Ledoux[2], Binui V. Taku[1], Mbouh Mariama[1], Manfo T.F. Pascal[3], Nantia Akono Edouard[1]*

[1] *Department of Biochemistry, Faculty of Science, University of Bamenda, POBox 39, Bambili, Cameroon,*
[2] *Department of Biological Sciences, Faculty of Science, University of Bamenda, POBox 39, Bambili, Cameroon,*
[3] *Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, PO Box 63 Buea, Cameroon,*
** Correspondence should be addressed to AE Nantia*

Abstract
Background: *Termitomyces letestui is an edible mushroom that grows symbiotically with termites. Though this species is present in Cameroon markets, few investigations have been carried to clearly assess its nutrient content. This study aimed to evaluate the effect of collection areas, growth stage and body fruit parts on T. letestui macronutrients and some antioxidants.*
Materials and Methods*: Mushroom samples at different growth stages were collected or purchased from the local markets of three localities: Mbouda,* Njingoumbe *and Ndop from both the West and North West regions. Their lipid, carbohydrate, vitamin C, total phenols, flavonoid and ash contents were evaluated.*
Results*: The lipid contents were higher in Ndop samples than samples from the other 2 localities. Carbohydrate contents were generally steady over developmental stages, but lipids showed a stage decreasing pattern with the mushroom maturation process. T. letestui from the 3 localities contained all screened antioxidant compounds.The total phenolics and flavonoids were generally higher at the mushroom maturation or developmental stage 3, while there was a relative richness of phenolics in stipe and flavonoids in the cap. The ash content in mushroom samples was variable as function of the localities and the highest level was recorded at the mushroom developmental stage 3.****Conclusion:*** *This study demonstrated variability of T. letestui nutrients as function of the localities, fungal parts and developmental stages, with the mushrooms from Mbouda being generally richer in macronutrients and antioxidants.*

Key words*: antioxidants, collection areas, fruit body parts, nutrients, developmental stage, Termitomyces letestui*

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I. Introduction

Mushrooms are macro fungi with distinctive fruiting body which can be either epigeous or hypogenous, large enough to be seen with the naked eye, and can be picked with hand. They lack chlorophyll and consequently cannot use solar energy in manufacturing their food [1]. According to their uses, mushrooms can be broadly grouped into three categories namely edible, medicinal and poisonous [2]. Thanks to their unique nutrients across plant- and animal-based food groups, mushrooms contribute significantly to human food security, especially in developing countries [3,4]. Nutritionally, mushrooms are low in energy and fats but high in proteins, carbohydrates, and dietary fibres. Mushrooms contain a variety of minerals and trace elements such as potassium, copper and vitamins including riboflavin, niacin, and folates. They have been used as food for centuries because of their unique taste. Apart from being recognized as a nutritious food, certain mushrooms are also an important source of biologically active compounds with potential medicinal value. Bioactive secondary metabolites found in mushrooms include phenolic compounds, sterols and triterpenes [5]. Mushrooms have also become an attractive source for the development of drugs and nutraceuticals [6].

Termitomyces is a genus of mushrooms with seasonal fructifications and are found exclusively in certain regions of Africa and Asia [7]. *Termitomyces* species grow symbiotically with termites and are usually found growing on termite mounds or in the vicinity of the mounds [8,9]. This genus includes many edible species

among which *Termitomyces letestui* (Pat.) R. Heim which is one of the biggest gill mushrooms in tropical Africa and has a good nutritional value compared to other mushrooms [10]. This species is recognized mainly by its large and fleshy pileus (cap) with a characteristic mammillate perforatorium and by the sheathing annulus on the long and conspicuous stipe that is easily separable from the cap. During its growth, it exhibits various sizes and shapes from the small young globose to subglobose cap to the large old (mature) expanded applanate pileus through the medium convex form. These morphological changes may have influence on the amount and distribution of nutrient contents of this species according to growth stage and fruit body part [7].

Due to its good taste, *T. letestui* is a highly prized food in many areas of tropical Africa where it is offered for sale in local markets and on roadsides [11]. In Cameroon, it is distributed in almost all agro-ecological zones especially in the savanna areas. In the western highlands of Cameroon, it grows at the beginning of the rainy season from March to May and is used by locals as food or income source [12]. Some locals of this area have different appreciations of this species according to its growth stages or fruit body parts. Though some studies have assessed the nutrient contents of *T. letestui* [10,13,14], no investigation has been carried out to address the variation of its nutrient content according to the growth stage, basidiomata part or collection area for a better understanding of its actual nutritive importance and promote its use as food entrant. This study therefore aimed to evaluate the effect of collection areas, growth stage and fruit body parts on *T. letestui* macronutrients (lipids, carbohydrates), antioxidants (vitamin C, total phenols, flavonoids) and ashes.

II. Materials and methods

Mushroom collection and treatment

Mushroom samples at different developmental stages (egg, elongation and maturation) were bought from the local markets of Mbouda city (Bamboutos division, West region of Cameroon), Njingoumbe village (Noun division, West region of Cameroon) and Ndop (Ngo-Ketunjia division, North-West region of Cameroon) in April 2018. The identification was confirmed by macroscopic and microscopic characteristics and using literature on mushrooms in Africa [15,16]. Different growth stages were carefully selected as follow: young basidiomata with small globose and closed pileus (egg stage or stage 1), medium basidiomata with convex early opening pileus (elongation stage or stage 2) and mature or old sporaocarps with large totally opened applanate pileus (mature stage or stage 3) (figure 1). They were cleaned, weighed and dried until having a constant mass using a legume fruit dryer at 43°C.

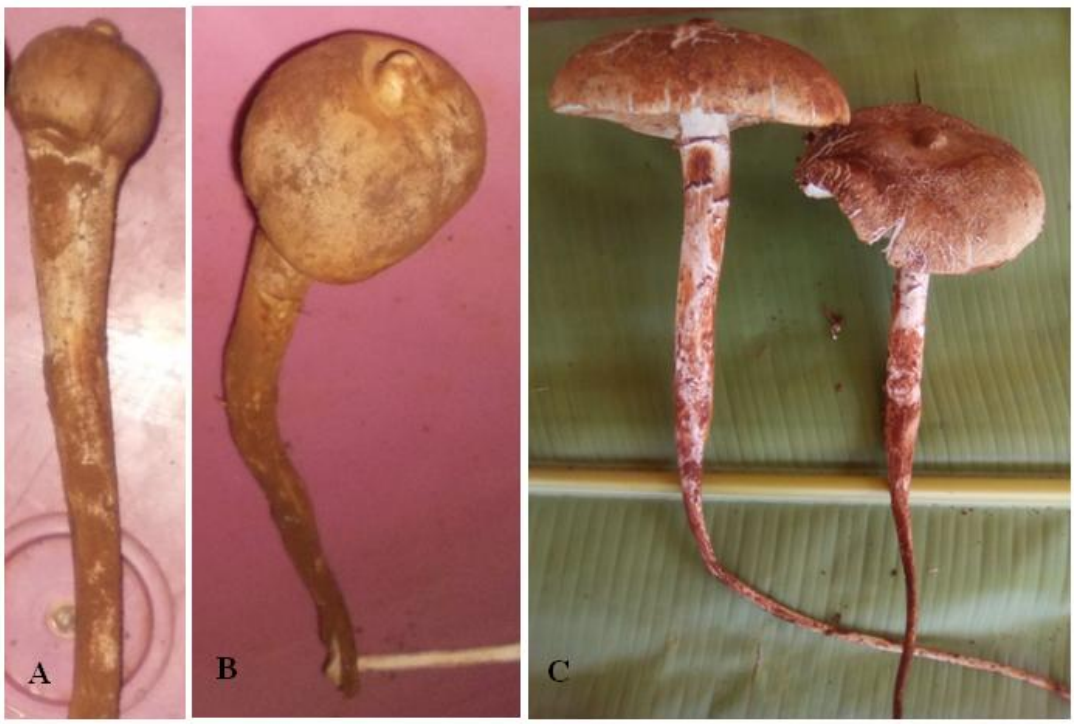

Figure 1: *T. letestui* at different growth stage, egg stage or stage 1 (A), elongation stage or stage 2 (B), mature stage or stage 3 (C)

Chemicals

Potassium dihydrogen phosphate and ethanol were obtained from Guandong Guanghua Sci-Tech Co. Ltd (Guandong, China), while Methanol, phenol and sulphuric acid were purchased from Riedel-de Haen (Bucharest, Romania), Kernel (Tianjin, China) and Loba Chemie Pvt. Ltd. (107, Woodehouse, Mumbai, India), respectively. Folin Ciocalteu's (FC) reagent was procured from Qualigens Fine Chemicals (Bombay, India). Quercitin and gallic acid were gotten from Griffin and George (Wembly Middlesex, England). All other chemicals were of analytical grade.

Quantification of total carbohydrates

The total carbohydrate content was quantified using the phenol – sulphuric acid method as described by Agrawal et al. [17]. Briefly, the dried and powdered samples were extracted using 70% ethanol. Two milliliters of the extract (or 2 mL of phosphate buffer for the blank tubes) were mixed with 1 mL of concentrated sulphuric acid into a test tube. The mixture was incubated at 40°C for 15 min and 0.2 mL of 6% phenol was added. The tubes were homogenized for 30 sec and incubated at room temperature for 20 min for colour development and absorbance measured at 580 nm using a UV-vis spectrophotometer. The concentrations of carbohydrates in samples were determined using standard curve obtained from glucose (1 to 5 mg/ mL).

Estimation of vitamin C

The total vitamin C content was analysed by redox titration using iodine as described by Ikewuchi and Ikewuchi [18]. Briefly, 4 mL of mushroom extract was introduced in a conical flask followed by 16 mL of distilled water and 5 drops of starch solution (0.5%). After homogenization the mixture was titrated with 5 mM iodine solution till color change (first permanent blue- black coloration) was observed. The concentration of vitamin C in the samples was calculated using the titer volume of iodine and vitamin C standard (5 mg/mL).

Quantification of total phenolic compounds

The total phenolic content of the mushroom extract was estimated by Folin Ciocalteu's method as described by Nantia et al. [19] with slight modification. In test tubes containing 0.2 mL of mushroom extract, 0.9 mL of H_2O and 0.1 mL of Folin Ciocalteu reagent were added. The mixture was homogenized and incubated at room temperature for 10 min, and 1 mL of 20% Na_2CO_3 and 2.5 mL of distilled water were added. The mixture was homogenized and incubated for 2 hours for colour development, and absorbance read at 750 nm using a UV-visible spectrophotometer. The total phenolic content of the extract was expressed as mg of gallic acid equivalent per mass of extract (mg GAE/ 100 g of extract) using standard gallic acid calibration graph (concentrations: 2.0 to 22.0 µg/mL).

Quantification of flavonoids

The mushroom content in total flavonoid content was determined by the aluminum chloride colorimetric assay using quercetin as the standard [19]. Briefly, 0.2 mL of mushroom extract and 0.1 mL of 1 M potassium acetate were mixed into a test tube. Aluminum chloride solution (10%, 0.1 mL) was added and the mixture incubated at room temperature for 30 min for colour development. Thereafter, 2ml of distilled water were added into the tube and the absorbance recorded at 415nm using a UV – visible spectrophotometer. The total flavonoids content of samples was expressed as µg of quercetin equivalents per mass of extract (µg QE/ 100 g of extract) using a quercetin calibration curve (concentrations: 0.3 to 3.4 µg/mL).

Determination of ash content

Five grams of the mushroom samples were placed into crucibles, and the sample incinerated at 550°C for 6 hours. Crucibles were later removed and kept in the desiccator (containing silica gel) to cool down without absorbing moisture. The dried crucibles were weighted using an electronic balance and the weight noted. The ash content was determined as percentage using weight of fresh sample and that of the ash sample.

Statistical analyses

Data were presented as means ± standard deviations. Differences between means were assessed using analysis of variance (ANOVA), and pairwise comparisons done using the Student Newman Keuls' test. All analyses were performed using MedCalc v14.8.1.

III. Results

Lipid content

The Ndop samples showed the highest ($P< 0.05$) total lipid content as compared to the samples from Mbouda and Njingoumbe localities (Table 1). In general, the lipid content in mushroom cap or stipe of each locality showed variable amount respective to the developmental stages. There was no difference of this nutrient between the mushroom stipe and cape. Regarding the developmental stages, highest lipid content was obtained

at stage 1 in mushroom from Mbouda and Njingoumbe, and at stage 2 in Ndop samples as compared with other stages.

Table 1: Total lipid content in mushroom parts from the studied localities.

Locality	Mushroom part	Lipids (g/100g)			
		Stage 1	Stage 2	Stage 3	Lipids per locality
Mbouda	Cap	1.30±0.42	1.00±0.00	1.20±0.57	1.12±0.34
	Stipe	1.90±0.14*	0.70±0.14	1.40±0.28*	1.33±0.64
	Cap & stipe	**1.60±0.59**	**0.85±0.19**	**1.30±0.38**	**1.25±0.50**
Njingoumbe	Cap	3.28±0.28*	2.00±0.00*	0.40±0.00	1.80±1.18
	Stipe	3.80±0.28*	2.20±0.28*	0.80±0.28	2.27±1.36
	Cap & stipe	**3.40±0.52^{c}**	**2.10±0.20^{b}**	**0.60±0.28^{a}**	**2.03±1.24**
Ndop	Cap	2.60±0.28	4.20±0.28*	4.50±0.14*	3.77±0.93
	Stipe	2.30±0.42	3.60±0.28*	2.60±0.28	3.10±1.16
	Cap & stipe	**2.45±0.34^{a}**	**4.30±0.68^{b}**	**3.55±1.11^{a}**	**3.43±1.06***

Data represent mean ± SD of 3 independent experiments. Differences among the same mushroom fruit body part as function of stages are presented with *. Other differences are illustrated with letters. Data with different letters within the same row or column are significantly different, P< 0.05 (Student Newman Keuls test).

Carbohydrates

Soluble carbohydrates in mushroom samples from the 3 different localities are presented in table 2. The samples from Ndop generally showed the lowest though not significant total carbohydrates as compared with samples from the other two localities. Carbohydrates in mushroom cap or stipe from each locality displayed inconsistent variation according to the growth stages, though there was higher content of this nutrient in the cap than stipe at stage 1. Only Njingoumbe samples displayed stage-dependent decrease of carbohydrate levels with significant (P< 0.05) effect observed at stage 2 and 3. In general, mushroom parts from each locality displayed comparable carbohydrates levels.

Table 2: Carbohydrate content in mushroom parts from the studied localities.

Locality	Mushroom part	Carbohydrates (g/100g)			
		Stage 1	Stage 2	Stage 3	Carbohydrates per locality
Mbouda	Cap	25.90±0.45*	19.95±0.68	26.45±0.68*	24.10±3.61
	Stipe	21.45±2.49	27.65±2.04	21.70±1.36	23.60±3.51
	Cap & stipe	**23.68±3.15**	**23.80±5.45**	**24.08±3.36**	**23.85±0.35**
Njingoumbe	Cap	24.35±2.04*	19.10±2.26	17.45±2.04	20.30±3.60
	Stipe	22.85±1.58	28.05±2.49*	19.50±3.17	23.47±4.31
	Cap & stipe	**23.60±1.06^{b}**	**23.58±6.33^{a}**	**18.48±1.45** ^{a}	**21.88±2.24**
Ndop	Cap	26.90±2.26*	21.75±1.13	20.10±2.26	22.92±3.55
	Stipe	12.45±1.58	14.20±0.91	19.95±0.68*	15.53±3.92
	Cap & stipe	**19.68±10.22**	**17.98±5.34**	**20.02±0.11**	**19.23±5.22**

Data represent mean ± SD of 3 independent experiments. Differences among the same mushroom fruit body part as function of stages are presented with *. Other differences are illustrated with letters. Data with different letters within the same row or column are significantly different, P< 0.05 (Student Newman Keuls test).

Vitamin C

In general, the vitamin C content of *T. letestui* showed no significant variation among localities, though there was a tendency of low amount in samples from Ndop (table 3). Within sample from each locality, vitamin C levels were higher in cap and stipe at stages 1 and 3, respectively in mushrooms from Njingoumbe and Ndop. The level of this micronutrient did not vary according to the developmental stages of the mushroom parts.

Table 3: Vitamin C content in mushroom parts from the studied localities.

Locality	Mushroom part	Vitamin C (µg/g)			
		Stage 1	Stage 2	Stage 3	Vitamin C per locality
Mbouda	Cap	10.0±2.8	10.0±2.8	12±0.0	10.7±2.1
	Stipe	8.0±0.0	10.0±2.8	8.0±0.0	8.7±1.6
	Cap & stipe	**9.0±2.0**	**10.0±2.3**	**10.0±2.3**	**9.7±2.1**
Njingoumbe	Cap	12±0.0*	6.0±2.8	8.0±0.0	8.7±3.0
	Stipe	6.0±2.8	6.0±2.8	12.0±0.0*	8.0±3.6
	Cap & stipe	**9.0±3.8**	**6.0±2.3**	**10.0±2.3**	**8.0±3.2**
Ndop	Cap	8.0±0.0*	4.0±0.0	6.0±2.8	6.0±2.2
	Stipe	4.0±0.0	8.0±0.0	6.0±2.8	6.0±2.2
	Cap & stipe	**6.0±2.3**	**6.0±2.3**	**6.0±2.3**	**6.0±2.1**

Data represent mean ± SD of 3 independent experiments. Differences among the same mushroom fruit body part as function of stages are presented with *. Other differences are illustrated with letters. Data with different letters within the same row or column are significantly different, P< 0.05 (Student Newman Keuls test).

Phenolic compounds

The phenolic content of *T. letestui* samples did not vary significantly between different localities though lower levels were observed in Ndop samples (table 4). Phenolics were in general abundant in cap at stage 1 and 2, while in stipe they were higher at stage 2 and 3. Out of the three localities, only Ndop samples showed stage development variation with high (P< 0.05) phenolic levels at stages 2 and 3. Similarly, only Njingoumbe displayed difference in phenolic content between the mushroom parts with more of this phytonutrient (P< 0.05) found in stipe as compared to the cap.

Table 4: Phenolic contents in mushroom parts from the studied localities.

Locality	Mushroom part	Phenols (µg GAE//g)			
		Stage 1	Stage 2	Stage 3	Phenolics per locality
Mbouda	Cap	60.80±3.03*	77.01±3.22*	43.51±7.01	60.44±15.43
	Stipe	72.32±1.14*	59.46±7.20	76.61±0.38*	69.46±8.62
	Cap & stipe	**66.56±6.91**	**68.23±11.11**	**60.06±19.53**	**64.95±12.82**
Njingoumbe	Cap	47.94±2.84	47.94±1.90	46.33±4.17	46.01±3.05[a]
	Stipe	54.37±4.17	65.75±1.71*	68.70±0.95*	62.94±7.08[b]
	Cap & stipe	**49.07±6.77**	**56.84±10.39**	**57.51±13.15**	**54.48±10.25**
Ndop	Cap	30.65±0.95	37.89±10.42	43.11±1.52*	37.22±7.33
	Stipe	17.79±1.33	53.03±0.38*	56.11±3.60*	42.31±19.12
	Cap & stipe	**24.22±7.48[a]**	**45.46±10.61[b]**	**49.61±7.83[b]**	**39.76±14.06**

Data represent mean ± SD of 3 independent experiments. Differences among the same mushroom fruit body part as function of stages are presented with *. Other differences are illustrated with letters. Data with different letters within the same row or column are significantly different, P< 0.05 (Student Newman Keuls test). GAE: gallic acid equivalent.

Flavonoids

Flavonoid levels in *T. letestui* samples were similar among the 3 localities (Table 5). In sample from each locality, the mushroom growth stages variably affected the flavonoid contents in cap or stipe. According to the stage of development, stages 1 and mainly stage 3 of the Mbouda and Njingoumbe mushrooms showed the highest (P< 0.05) flavonoid content as compared to the other stages. Within locality, only mushroom samples from Mbouda showed higher flavonoid levels in cap than stipe.

Table 5: Flavonoid levels in mushroom parts from the studied localities.

Locality	Mushroom part	Flavonoids (µg QE/g)			
		Stage 1	Stage 2	Stage 3	Flavonoids per locality
Mbouda	Cap	125.71±14.98*	67.79±0.64	149.37±3.83*	114.29±38.17^{b}
	Stipe	60.58±5.74	56.52±2.55	37.14±1.28	51.41±11.56^{a}
	Cap & stipe	**93.14±38.73** ^{b}	**62.16±6.68**^{a}	**93.26±64.84**^{b}	**82.85±42.44**
Njingoumbe	Cap	133.60±3.83*	45.25±3.83	119.85±11.79*	99.57±42.91
	Stipe	68.47±0.32	88.07±0.64*	111.06±21.04*	89.20±21.27
	Cap & stipe	**101.03±37.67**^{ab}	**66.66±24.82**^{a}	**115.46±14.82**^{b}	**94.38±32.74**
Ndop	Cap	52.24±6.06	102.72±2.23*	59.45±0.96	71.47±24.59
	Stipe	97.99±14.02*	67.11±16.26	47.06±5.10	70.72±24.98
	Cap & stipe	**75.11±27.85**	**84.92±22.64**	**53.25±7.76**	**71.10±23.64**

Data represent mean ± SD of 3 independent experiments. Differences among the same mushroom fruit body part as function of stages are presented with *. Other differences are illustrated with letters. Data with different letters within the same row or column are significantly different, P< 0.05 (Student Newman Keuls test). QE: quercetin equivalents.

Ash content

Mushroom samples displayed variable content in ash as function of the localities with Ndop samples having the highest ash content (Figure 2). In general, the ash content of the mushroom samples showed a stage development dependent increase. Mushrooms from Mbouda contained higher amount of ash at stage 3 (P< 0.05) as compared with stages 1 and 2.

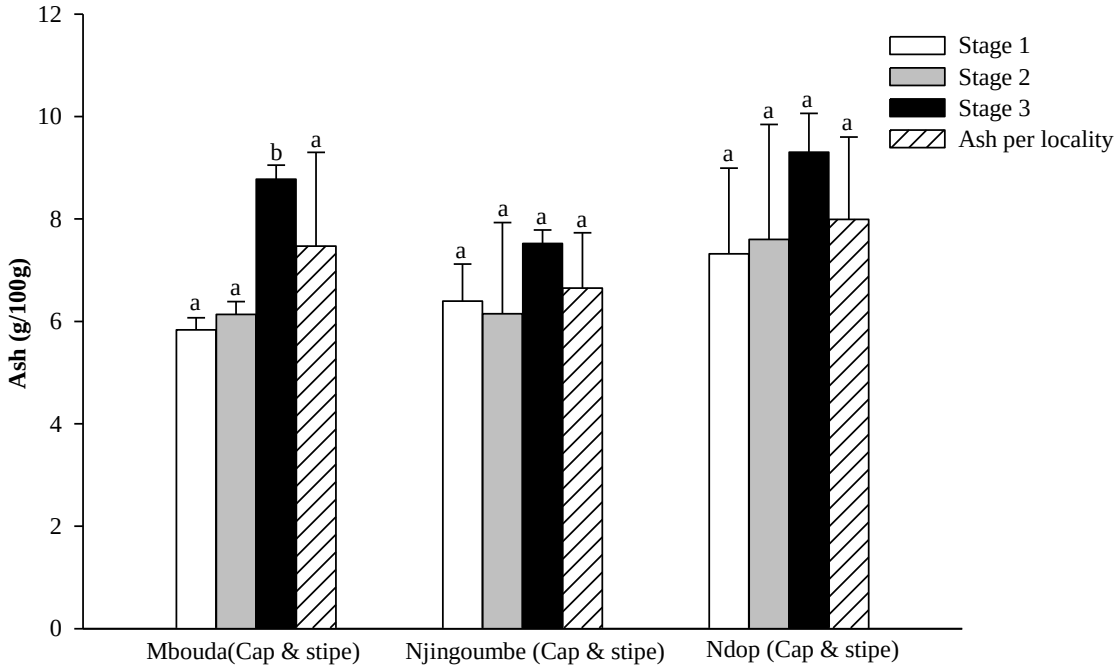

Figure 2: ash content in mushroom samples from different localities.

Data represent mean ± SD of 3 independent experiments. Data with different letters within bars are significantly different, P< 0.05 (Student Newman Keuls test).

IV. Discussion

Mushrooms have gradually become valuable sources of both micronutrients and macronutrients [4,5]. As plant diversity varies from one area to another with plant species that have different constituencies, samples of *Termitomyces* species from various localities may show some differences in their nutrient composition. Though our localities are rich in different mushrooms, several species have not been investigated for their actual content in nutrients. In the present study we evaluated the effect of collection areas, growth stage and sporophore parts on the mushroom *T. letestui* macronutrients (lipids, carbohydrates), antioxidants (vitamin C, total phenols, flavonoids), and ashes.

Foods with low fat calories are of high interest for human health [20]. Mushrooms from Mbouda and Njingoumbe localities showed low lipid content as compared to the Ndop samples, but the obtained lipid proportion were within ranges of 20–30 g/kg reported previously [21]. The low lipid content in *T. letestui* could be interpreted as the low fat calorie content of Mbouda and Njingoumbe mushrooms, though the most essential nutrients of lipids are essential fatty acids which must be obtained from dietary plant sources and play vital biological functions including component of plasma and erythrocyte membranes, metabolic fates and precursors of prostaglandins. Consequently, deficiencies in essential fatty acids are associated with oxidative stress, inflammatory, metabolic and cardiovascular diseases as well as skin disorders and poor growth [22]. The present study however did not focus on the actual composition of *T. letestui* in essential fatty acids that could be considered in our future investigations. Regarding the developmental stages, the highest lipid content was concentrated in the 2 first stages and this illustrates a certain decrease of the total lipids with the maturation process of *T. letestui*. However Ahlawat and Tewari [23] reported that fat content increases with the maturation stage. The inconsistency with our findings could be explained by the fact that we considered the total lipids including fats in the present investigations.

Carbohydrate levels did not vary according to the localities neither regarding the mushroom parts considered. However there was a stage- dependent decrease of carbohydrates from stage 1 to stage 3. This result is in line with earlier reports that showed that carbohydrates increase from egg stage to the elongation stage levels and drops at the mature stage [23]. The presence of carbohydrates mushrooms further supports some health-promoting properties and biological activities as polysaccharides produced by different varieties mushrooms are a source of prebiotics (that have the potential to promote human health). The latter are defined as selectively fermented food ingredients that induce changes in the composition and activity of the gastrointestinal tract microbiota that confer nutritional and health benefits to the host. Such compounds include mushroom polysaccharides, most of which are β-glucan polymers, chitin, mannans, galactans and xylans [24].

Ash content was not significantly different among the localities, but regarding stages, mushrooms from Mbouda showed higher ash content at stage 3, though in general within the ranges of 5 – 17% recorded from previous studies [21,25,26]. Ash refers to inorganic residue remaining after either ignition or complete oxidation of organic matter in a foodstuff. Ash content represents the total mineral content in foods [27]. The nature of soil and the substrate of the investigated localities likely have no significant influence on the total mineral of *T. letestui*. The high ash content noted in mushroom samples from Mbouda at the developmental stage 3 or the maturity stage could be to some biotic factors such as substrates though according to some reports ash content remains almost similar at all the developmental stages [23,28].

Besides macronutrients, an important portion of mushroom nutritive and medicinal properties is thanks to the content in micronutrients and phytochemicals having antioxidant ability [29,30]. *T. letestui* from the 3 localities contained appreciable amount of the screened phytonutrients, vitamin C, phenolic compounds and flavonoids. Vitamin C is one of the essential vitamins only supplied in human through diet. It plays vital physiological functions including metabolic functions (activation of the B vitamin and folic acid, conversion of cholesterol to bile acids, conversion of tryptophan to the neurotransmitter, serotonin), development and maintenance of connective tissues, protection of the immune system and reduction of the severity of allergic reactions. It is an efficient soluble plant antioxidant that protect from the oxidative damage through its reversible oxidization to ascorbyl radical and then to dehydroascorbate [31,32]. Flavonoids and the other phenolic compounds are plant secondary metabolites generated by plant to defend itself or to promote the growth under unfavorable conditions. They are characterized by an aromatic ring bearing at least one hydroxyl groups and half of these phenolic compounds are flavonoids presenting as aglycone, glycosides and methylated derivatives. Polyphenols are effective reactive oxygen species scavengers and metal chelators due to the presence of multiple hydroxyl

groups. They are effective protectors against development of many diseases including cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases [33,34]. Harvesting localities did not affect the screened antioxidant compounds. In general, total phenolics and flavonoids were higher at mushroom maturation or developmental stage 3, while there was a relative richness of phenolics and flavonoids in stipe and cap, respectively. The high antioxidant levels in mushrooms at the maturity is similar to the study of Barros et al. [35] who found elevated antioxidant contents at the mature stage of the mushroom *Lactarius piperatus*. On the hand, the variability of antioxidant properties according to the mushroom parts (cap, stipe) has been demonstrated in different studies [36,37]. The antioxidant content of *T. letestui* could justify various biological activities and ethnobotanical uses of this mushroom [9,38,39].

Conclusively, our findings demonstrated variability in *T. letestui* nutrients as function of the harvest locality, part and stage of development with the mushrooms from Mbouda being generally richer in macronutrients and antioxidants. This variation of the nutrient contents in *T. letestui* further illustrates a certain difference in raw material (wood, dry grass, leaf litter) used by termites to produce the comb. Future studies on *T. letestui* would help to define additional important nutrients including proteins, essential fatty acids and prevalent classes of phenolic compounds.

Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this manuscript.

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